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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 3 SEPTEMBER, 1945 No. 9

PLANT PROTEINS

Each species of plant seed contains a wide variety of protein components of different properties. As a rule, only a limited number of these, often only two or three, are present in relatively large proportions. These major components are relatively stable substances which are laid down in the endosperm as a source of nitrogenous nutriment for the development of the embryo during the earliest stages of growth. For the differentiation and characterization of the main proteins of a large number of the seeds used as food by man and the domesticated animals, we are indebted to Ritthausen and more especially to Osborne. The traditions established by these great pioneers have, however, been carried on by a small group of subsequent workers.

Although present day knowledge of the solubility and other physical properties, of the amino acid composition, and of the nutritive effect of a considerable number of individual seed proteins is fairly extensive, there is no one of these proteins for which the record of composition is complete, and there is no single seed from which more than a fraction of the protein components has been isolated in the form of moderately homogeneous preparations (Vickery, *Physiol. Rev.* **25,** *347 (1945)*). Even in the case of wheat, the most important seed of all, investigators have been unable to agree upon the most effective methods for the separation and purification of the main proteins, and astonishingly little is known about the subsidiary proteins (Bailey, "The Constituents of Wheat and Wheat Products," *Reinhold, New York (1944)*). Still less adequate is the information concerning the proteins of maize and rice and the other important cereals, or the large group of legume seeds commonly used as food.

Investigators have, for the most part, concentrated their attention upon the protein component that can be obtained in largest yield. The emphasis has usually been placed upon purification and reproducibility with the result that such cereal proteins as gliadin from wheat, zein from maize, and a few of the oil-seed globulins, notably edestin from hempseed, are moderately well characterized substances. The fact that these particular proteins represent one-half or less of the total protein of the seed from which they are derived is frequently overlooked. The same is true for the globulins of the legumes. Soybean globulin, for example, can be separated by dialysis, purified by reprecipitation, and obtained in a total yield of about 20 per cent from fat-free soybean meal that contains 7.0 per cent of protein nitrogen, and accordingly at least 40 per cent of protein. Globulin preparations obtained in this way have been studied in some detail, but practically nothing is known about the remaining half of the protein. Although the whole seed as well as the isolated main globulin have moderately good nutritive properties (Osborne and Mendel, *Z. f. physiol. Chem.* **80,** *307 (1912); J. Biol. Chem.* **32,** *369 (1917)*), it cannot be inferred that the remaining unknown proteins are likewise adequate with respect to the amino acids essential in nutrition. Clearly, therefore, an extension of our present knowledge of the nature and properties of seed proteins is much to be desired. To be sure, much of the cream has already been skimmed and startling new discoveries are not to be anticipated although it would perhaps be reckless to be too sure even of this. However, the fact that we are unable even today to write the composition of a single plant seed in terms much more detailed than those of the proximate chemical

analysis employed for generations by the agricultural chemist is a challenge to present day investigators. The plant seed is the ultimate source of all food for both man and animal and is thus one of the most important organisms with which man has to deal; it is time that we knew something about its composition in terms more exact than protein reckoned from the nitrogen content by means of an assumed factor, the ether extract, the starch and soluble carbohydrates, and the crude fiber.

Plant proteins used as food are by no means limited to the seeds; the green tissues of plants and many fleshy roots are likewise important sources. Unfortunately, however, the position of our knowledge of the proteins of the tissues of plants other than the seed is far less secure even than our knowledge of seed proteins. Proteins are present in all living cells, and have been specifically recognized in leaves, stalks, and roots (Jones and Gersdorff, *J. Biol. Chem.* **93,** *119* (*1931*)), as well as in fruits, in the bark (Jones, Gersdorff, and Moeller, *Ibid.* **64,** *655* (*1925*)), and in the juices and exudates of plants. There is a small literature on the proteins of roots and of fruits (e.g. Sinclair, Bartholomew, and Nedvidek, *J. Agr. Research* **50,** *173* (*1935*)) and, for example, that of the latex of the rubber plant (Tristram, *Biochem. J.* **34,** *301* (*1940*); **35,** *413* (*1941*)), but, for the most part, detailed investigation has been restricted to the leaf. The practical agriculturalist has always recognized the adequacy of the nutritive properties of the proteins of leaves; the production of livestock is founded upon this observation and, although differences in the quality of various fodder plants are fully appreciated, to what extent this is due to the composition of the proteins is unknown.

Students of nutrition have rarely devoted attention to growth experiments upon the proteins of leaves because of the experimental difficulty of isolating these proteins in a form suitable for such studies. Osborne in this country and Chibnall in England in 1920 were the first to attempt to do this; Osborne's investigations (Osborne and Wakeman, *J. Biol. Chem.* **42,** *1* (*1920*); Osborne, Wakeman, and Leavenworth, *Ibid.* **49,** *63* (*1921*)) were extended only far enough to demonstrate that moderately pure samples of representative leaf proteins could be secured, but Chibnall has pursued the problem for many years ("Protein Metabolism in the Plant," *New Haven* (*1939*)). He developed technics for the isolation of the proteins of the leaf cytoplasm and subjected these to amino acid analysis whereby results were obtained that led to a partial explanation at least of the high repute that these proteins have long held in the field of animal nutrition. The most striking observation was that the cytoplasmic proteins from the leaves of a wide variety of plant species have a closely similar amino acid composition.

Leaf cells, when examined under the microscope, are seen to be filled with a viscous jelly-like mass of cytoplasm that contains an enormous number of tiny formed elements called chloroplasts or plastids in which the green pigment is concentrated. Estimates as high as 400,000 of these per square millimeter of leaf surface have been made (Weier, *Bot. Rev.* **4,** *497* (*1938*)). It was early recognized that the chloroplasts contain protein as well as pigment, and the nature of the association between the protein and the chlorophyll in the chloroplast has long been an important problem. The work of Menke in Germany (*Z. f. physiol. Chem.* **257,** *43* (*1938*); **263,** *100, 104* (*1940*)), of Chibnall in England, and of Granick (*Am. J. Bot.* **25,** *558* (*1938*)) and Smith (*J. Gen. Physiol.* **24,** *565, 583* (*1941*)) in this country has contributed to our knowledge on this matter in recent years. Methods for the isolation of the chloroplasts have been developed, and it has been established that they contain from 35 to 45

per cent of the protein of the leaf as well as all of the chlorophyll and yellow pigments, together with other lipids. When treated with hypotonic solutions, the chloroplasts swell and discharge their contents in the form of green granules that pass into a colloidal solution which shows a purely random distribution of particle size. The pigment is present in what gives every evidence of being chemical combination with the protein that makes up a large part of these granules. When treated with detergents, the colloidal solution becomes brilliantly clear, and Smith and Pickels have shown (*J. Gen. Physiol.* **24,** *753 (1941)*), by means of the ultracentrifuge, that the chemical combination between chlorophyll and protein is not broken if sodium dodecyl sulfate is used; the protein and the pigment sediment together in the gravitational field. With other detergents, the combination is disrupted; the pigment remains in solution but the protein sediments at a rate consistent with the view that it is homogeneous with respect to molecular size and possesses a molecular weight that is estimated to be of the order of 265,000. Both green and yellow pigments appear to be associated with this protein, and the inference may thus be drawn that the chloroplast contains a complex lipo-protein of high lipid content, probably of the order of about 30 per cent.

At the present time, then, methods for the isolation of the two main types of protein components of leaf cells have been developed, although these methods are admittedly crude and elementary and are still far from effective. Nevertheless, ground has been broken and the way is open for the detailed study of the physiologically active proteins of green plant tissues. Technics for the study of the lipo-proteins in general and especially those of blood serum are today being rapidly developed and our understanding of these vitally important substances is advancing. The leaf chloroplasts in which the complex chlorophyll lipo-protein is located, although technically classed as inclusions in the cytoplasm of the leaf cell, are in effect themselves tiny cells the dimensions of which are similar to those of the red blood cell. Although it is considerably more difficult to secure preparations of isolated chloroplasts for study than it is to isolate red cells, there is no obvious reason why many of the well developed technics of hematology should not be applied in this somewhat allied field. Thus the outlook for future progress in our knowledge of the proteins of leaves is indeed promising and the day is rapidly approaching when we shall be far better satisfied than we are at present with our knowledge of the proteins of plants.

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NUTRITIONAL ASPECTS OF PROGRESSIVE MUSCULAR DYSTROPHY

Progressive muscular dystrophy is one of a number of muscular dystrophies to which man is subject. It is commonly a fatal hereditary disease for which no satisfactory treatment has yet been found. Since muscular dystrophy is one of the major signs of vitamin E deficiency in experimental animals, considerable interest has been evidenced concerning possible relationships between vitamin E and progressive muscular dystrophy.

Shank, Gilder, and Hoagland (*Arch. Neurol. Psychiat.* **52,** *431 (1944)*) have made an extensive study of 40 cases of progressive muscular dystrophy. Although these workers had little success with therapy, they

have added to the knowledge of the nature of this disease. As had been previously observed, there was an increased rate of excretion of creatine and a decreased rate of excretion of creatinine in boys with muscular dystrophy as compared with normal boys. The differences were accentuated by fasting. The concentration of plasma creatine but not of plasma creatinine was higher in dystrophic children. Creatine tolerance was not significantly affected. Contrary to previous reports the basal metabolic rate was lower in children with progressive muscular dystrophy than in normal controls, being minus 14.5 per cent of normal standards. Glucose, nitrogen, and phosphorus metabolism were not implicated in this disorder.

Fourteen patients were treated with glycine, 5 were treated with glycine plus ephedrine, 3 were treated with gelatin, and others were treated with vitamins of the B group; none was helped. The same negative effects were found in those cases given alpha-tocopherol (vitamin E) or wheat germ oil.

In spite of these negative findings of Shank *et al.* a report by Milhorat and Bartels (*Science* **101,** *93 (1945)*) that tocopherol plus inositol decreases the creatinuria in dystrophic patients, has revived the question of a possible role of vitamin E in human dystrophy. It was observed that oil obtained by extraction of wheat germ with warm ethylene dichloride reduced the creatinuria of 4 patients. When the wheat germ was first defatted with ether and then extracted with ethylene dichloride, a preparation was obtained that was stated to be 15 times as effective as the original oil of the whole wheat germ.

It was subsequently found that tocopherol refluxed with ethylene chloride (or autoclaved with ethylene oxide at 200°) gave rise to a product which decreased creatinuria in 60 per cent of the patients with mild dystrophies. Heating tocopherol with benzene hexachloride in alkali yielded a product which even in high dilutions was effective in decreasing creatinuria. Since benzene hexachloride is a chloride substitution product of inositol, the authors assumed that a condensation product between tocopherol and inositol might be formed and that this product was responsible for the lowering of creatinuria. The hypothetical condensation product between tocopherol and inositol was described as being 2500 times as effective in reducing creatinuria as wheat germ oil obtained by ethylene dichloride extraction. In one patient with muscular dystrophy of "moderate severity" a single dose of 60 mg. of this product given with alkali reduced the creatinuria to one-half the control level in three days. The effect lasted about a week. The authors noted that in 4 other patients with rapidly progressive symptoms the effect of the compound was considerably smaller. Thus, in one of these subjects a single dose of 95 mg. of the product lowered the creatine output only 14 per cent for three days. Tocopherol or inositol given alone was without effect on creatinuria. However, the authors state that they obtained definite effects in 5 of the 7 patients when these two compounds were administered together in equimolar amounts for one or two days. The dosage of the mixture required to produce effects on creatinuria appeared to be proportional to the rate of progression of the dystrophic process.

The authors describe the mixture of tocopherol and inositol as being "from $\frac{1}{8}$ to $\frac{1}{30}$ as effective as the condensation product."

Incubation of the mixture with an extract of hog stomach and duodenum increased the effect. The authors also reported that incubation of tocopherol alone in the stomach of a normal man gave a product which was effective in lowering the creatine output of half of a group of dystrophic patients.

Milhorat and Bartels offer the following hypothesis in an attempt to interpret their findings. Tocopherol forms a condensation product with inositol in the gastrointestinal tract (tocopherol-inositol ether). The ability to synthesize this condensation

product has been lost in hereditary muscle dystrophy. Patients in whom the disease process is mild can synthesize sufficient amounts of the condensation product when large amounts of both tocopherol and inositol are given together. In rapidly progressing cases, however, the condensation product itself is required.

Although this report deserves considerable interest and attention, it is perhaps necessary to delay acceptance of the interpretations of the data until a more detailed account which can be studied critically has appeared. The authors state that investigation of the effect of inositol-tocopherol preparations on the clinical course of the disease is under way. The authors cite only one case in which a marked decrease in creatinuria occurred. The other cases cited showed, as pointed out by the authors, effects which were smaller and of shorter duration. One is perhaps justified therefore in questioning the statistical significance and the specificity of some of the changes.

At this stage, it is only possible to discuss the effects on creatinuria. These effects may or may not be related to the clinical course of the disease. It is known that creatinuria is by no means confined to diseases of muscle but is observed in a variety of physiologic and pathologic conditions. Hoagland, Gilder, and Shank (*J. Exp. Med.* **81,** *423 (1945)*) cite the following conditions under which creatinuria has been observed: starvation, carbohydrate privation, acidosis, high protein diets, and pathologic conditions such as malignancies, fever, thyroid, and parathyroid disease. They point out that the diminished excretion of creatinine in many cases gives a more reliable indication of the severity of the muscle dystrophia than does the increase in creatinuria. It would therefore be of great interest to know whether the hypothetical condensation product described above has any effect in increasing creatinine excretion.

LIVER FUNCTION AND EXPERIMENTAL CHOLINE DEFICIENCY IN DOGS

In a previous review (*Nutrition Reviews* **3,** *124 (1945)*) the studies of McKibbin, Thayer, and Stare (*J. Lab. Clin. Med.* **29,** *1109 (1944)*) on the experimental production of acute choline deficiency in young puppies were discussed. In that report, the development of a ration for the production of acute choline deficiency in weanling puppies and the impairment of liver function that resulted from the deficiency were described in some detail. Dutra and McKibbin (*Ibid.* **30,** *301 (1945)*) supply a description of the pathologic changes found in the same animals.

The livers of the deficient animals were pale yellow or pinkish yellow. They contained much lipid material in vacuoles which was distributed irregularly. The vacuoles varied from small to large, the latter being more numerous. The thymuses of these animals were invariably smaller than those of litter mate controls. In many instances there was no recognizable thymic tissue. Microscopically the thymuses differed from those of control animals in that there were few thymocytes in the medullary portions of the glands of the deficient animals and the stroma was, therefore, prominent. Other morphologic differences were noted but the most striking change was in the thymic corpuscles. In control animals these were relatively small and the cells comprising them had homogeneous cytoplasm and reticulated nuclei. In the deficient animals, they were represented by large cells which were often multinucleated and showed a number of morphologic abnormalities.

Animals receiving various amounts of choline chloride as a supplement were found

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Animals receiving various amounts of choline chloride as a supplement were found

to have changes in the liver corresponding to the quantity of choline chloride supplied. The livers of dogs which had received supplemental choline chloride in amounts of 10 mg. per 100 g. of ration were similar to those of animals which had received no supplemental choline. An animal which received 25 mg. of choline chloride per 100 g. of ration had appreciably less fat in its liver, and one which received 50 mg. per 100 g. of ration had a liver in which there were only small numbers of cells which contained visible fat vacuoles. The livers of animals which received suboptimal amounts of choline had relatively few cells which contained large fat vacuoles, but there were minute vacuoles of visible fat in nearly all the cells. The large vacuoles were in focal areas in the lobules and were central in some and peripheral in others. The liver was completely protected against fatty metamorphosis by 100 mg. of choline chloride per 100 g. of ration. Equal protection was afforded in the absence of choline by 0.7 g. of dl-methionine per 100 g. of ration. The livers of several deficient dogs were stained by acid-fat technic for ceroid but none of this substance was demonstrated. There was diminution of liver function, as measured by the levels of plasma phosphatase, cholesterol, and cholesterol esters, the prothrombin time and the bromsulfalein elimination, which correlated closely with the morphologic alterations and lipid contents of the livers of the deficient puppies.

The authors are of the opinion that more than simple fat infiltration occurs in the liver of choline deficiency. They reached this conclusion because of the fact that in pantothenic acid deficient puppies they found little functional abnormality of the liver even though 50 per cent of the dry weight was lipid material, whereas in the choline deficient animals marked changes in liver function were observed. They point out that it does not seem probable that simple fat infiltration could be responsible for marked changes in function in an organ known to have a large factor of safety.

In extension of these studies, McKibbin *et al.* (*J. Lab. Clin. Med.* **30**, *422* (*1945*)) were led to investigate tests for hepatic impairment based on testing the methyl reserves rather than by the customary procedures, since the latter are not specific but reflect parenchymal liver disease of varied etiology. In choosing the specific tests for methyl reserves, these investigators considered the fact that labile methyl derived from methionine or choline is concerned in the synthesis of creatine and creatinine in the rat, rabbit, and dog (du Vigneaud, *Harvey Lectures* **38**, *39* (*1942–43*)), and the possibility that other nitrogen or sulfur linked substances containing methyl groups, for example the N-methylated derivatives of nicotinic acid, are also dependent on dietary choline or methionine for their formation. Hence observations were made on the excretion of creatinine, creatine, and N-methylated derivatives of nicotinic acid by choline deficient and control puppies, as well as observations on the improvement in liver function in choline deficient animals following choline therapy.

Control animals receiving choline showed normal results on the customary liver function tests. Dogs given a diet lacking choline were found to have marked impairment of liver function by these methods, which returned to normal promptly on administration of choline. Thus, after a deficiency lasting sixty-eight days, the administration of 200 mg. per cent of choline chloride was associated with a return to normal liver function in ten days. In this period of time the plasma phosphatase dropped from 1348 to 357 Bodansky units, bromsulfalein retention was reduced from 38 per cent to 7 per cent, the total plasma cholesterol rose from 52 to 154 mg. per cent, the plasma cholesterol esters from 34 to 101 mg. per cent, and the prothrombin time returned to normal. Of all the indexes of liver function, the phos-

phatase level was the slowest to return to normal. In an animal sacrificed at this time the liver was found to contain a normal amount of lipid material as determined by chloroform extraction. At the same time striking increases in food consumption and body weight were produced by the choline supplements.

The addition of choline to the diet did not, however, produce any significant increases in the daily per kilogram output of creatinine or in the excretion of methylated nicotinic acid derivatives in terms of the per cent of the total nicotinic acid intake. One animal did show a significant increase in the per kilogram excretion of creatine after five days of choline supplementation. This was not observed in other animals.

The excretion of creatinine was found to be constant in all the dogs, whereas the output of creatine varied greatly between different dogs and considerable daily variation in the same animal was observed. Glycocyamine fed orally or injected subcutaneously did not appreciably increase either the creatine or creatinine output in 2 dogs. It seems clear from these studies that creatinine and probably creatine are independent of dietary choline.

These observations are disappointing in that they raise doubt as to the possibility of testing the methyl reserves of dogs by observations of creatine or creatinine excretion, or the excretion of methylated nicotinic acid derivatives following doses of nicotinic acid. The possibility still remains that other substances which depend on labile methyl from choline or methionine for their formation may be useful for the purpose of testing the methyl reserves.

A curious finding, difficult to explain, was that spontaneous improvement occurred in 2 dogs. The animals in which these spontaneous remissions occurred were the largest dogs of the litters. It is suggested that the choline requirement may be lowered after a certain stage in growth or development has been reached. The healing of kidney lesions in young rats surviving choline deficiency may be an analogous phenomenon.

THE END PRODUCTS OF NICOTINIC ACID METABOLISM

The subject of nicotinic acid metabolism was discussed within the past year (*Nutrition Reviews* **2,** *234* (*1944*)) but continued progress and interest in this field has led Ellinger (*Nature* **155,** *319* (*1945*)) to review it again. His (*Arch. f. exper. Path. u. Pharmakol.* **22,** *253* (*1887*)) reported that pyridine was converted by methylation in the animal body to N-methyl pyridinium hydroxide, i.e. to the N-methyl pyridinium ion. Ackermann (*Z. Biologie* **59,** *17* (*1912*)) a quarter of a century later, fed nicotinic acid to a dog and from its urine isolated 1.9 g. of nicotinuric acid (nicotinoyl glycine) and 1.5 g. of trigonelline (N-methyl-pyridinium-3-carboxylic acid). Komori and Sendju (*J. Biochem.* (*Japan*) **6,** *163* (*1926*)) reported that dogs excrete nicotinic acid as nicotinuric acid, as trigonelline, and also unchanged; that rabbits excrete nicotinuric acid but not trigonelline; and that birds excrete all nicotinic acid unchanged.

Little further attention was paid to these compounds until interest in nicotinic acid was revived by the discovery of its presence in the phosphopyridine nucleotides and its function as a vitamin. Two groups of workers (Sarett, Perlzweig, and Levy, *J. Biol. Chem.* **135,** *483* (*1940*); Melnick, Robinson, and Field, *Ibid.* **136,** *145* (*1940*)) then independently observed the presence of what they believed to be trigonelline in human urine. A quantitative method was developed for its measurement, depending on an incomplete transformation to nicotinic acid (Perlzweig, Levy, and Sarett,

Ibid. **136,** *729 (1940)*). This method, although laborious and not of the highest accuracy, was employed in the study of nicotinic acid metabolism in various species. When it was found that the substance measured as trigonelline was the chief end product of nicotinic acid metabolism in man, attempts were made to devise tests, based on its measurement, which would give information on the sufficiency or deficiency of nicotinic acid intake of the individual. These tests were rendered doubly difficult by the circumstance that trigonelline itself was known to occur in coffee, in legumes, and other foods, so that these items had to be excluded from the diet before making the test. Following a dose of nicotinic acid or amide an increase in the excretion of trigonelline was noted, which was greater in well nourished than in less well nourished subjects. Such a test for nicotinic acid deficiency was suggested by Field, Melnick, Robinson, and Wilkinson (*J. Clin. Invest.* **20,** *379 (1941)*) and was studied more extensively by Perlzweig, Sarett, and Margolis (*J. Am. Med. Assn.* **118,** *28 (1942)*).

More recently it has become clear that the compound which appears in increased quantity in the urine following the ingestion of nicotinamide or nicotinic acid is actually not trigonelline, but is the corresponding methyl derivative of nicotinamide. This compound has been variously named N′-methylnicotinamide (Huff and Perlzweig, *Science* **97,** *538 (1943)*; *J. Biol. Chem.* **150,** *395 (1943)*) and nicotinamide methochloride (Ellinger, *loc. cit.*), although a chemically precise name would be the N-methyl-pyridinium-3-carboxylic acid amide ion. This substance has been recognized following the discovery by Najjar and Wood (*Proc. Soc. Exp. Biol. Med.* **44,** *386 (1940)*) that the extraction of alkaline urine by butanol gives a fluorescent solution, and that the intensity of its fluorescence is increased following the ingestion of nicotinic acid or amide. The compound which fluoresces in butanol was named F_2 by Najjar and Holt (*Science* **93,** *20 (1941)*), but unfortunately the loose use of this name to designate sometimes the compound whose fluorescence is measured, and at others the actual metabolite excreted, has given rise to some confusion.

The nature of the actual metabolite was established by Huff and Perlzweig (*Science* **97,** *538 (1943)*; *J. Biol. Chem.* **150,** *395, 483 (1943)*) as the quaternary ammonium ion, N-methyl pyridinium-3-carboxylic acid amide, which they designated N′-methylnicotinamide. They isolated it from urine as the chloride and showed it to be identical with synthetic N′-methylnicotinamide chloride. Ellinger continues to refer to the metabolite as nicotinamide methochloride, which is less desirable if one accepts the usage of referring to the excretion of ammonia rather than a particular ammonium salt.

Data were presented by Huff and Perlzweig showing that after an oral dose of nicotinamide the measurement of the excreted metabolite as "trigonelline" and as N′-methylnicotinamide gave quantitatively closely similar results, indicating that the substance which earlier had been estimated as trigonelline actually was N′-methylnicotinamide. This identification of the metabolite has been questioned by Najjar and White (*Science* **99,** *284 (1944)*; *Ibid.* **100,** *247 (1944)*) and Najjar, White, and Scott (*Bull. Johns Hopkins Hosp.* **74,** *378 (1944)*) who have stated that the metabolite is N′-methylnicotinamide alpha-carbinol and that the fluorescent compound actually measured is the butyl ether of this carbinol. It is to be expected that the N-methyl-pyridinium hydroxide 3-carboxylic acid amide ion and the carbinol would be tautomers in dynamic equilibrium, so that the two structures both represent the compound, which exists as a mixture of the two forms in proportions which vary according to conditions of solvent, pH, etc. Either form could then be isolated, depending on

the reagents employed. In normal urine the water solvent and the pH would favor a preponderance of the N′-methylnicotinamide form, with only traces of the carbinol present. The identity of the fluorescing compound measured in the estimation remains uncertain.

The observation has been recorded by Najjar *et al.* (*Bull. Johns Hopkins Hosp.* **74,** *406* (*1944*)) that N′-methylnicotinamide chloride cures canine blacktongue, and these authors suggested that the essential nucleus of the pellagra-preventive vitamin is N-methyl-pyridinium-3-carboxylic acid. However, the earlier work of Woolley, Strong, Madden, and Elvehjem (*J. Biol. Chem.* **124,** *715* (*1938*)) as well as the later study of Teply, Krehl, and Elvehjem (*Proc. Soc. Exp. Biol. Med.* **58,** *169* (*1945*)) failed to confirm that this compound is effective in blacktongue, and in view of the large doses employed by Najjar *et al.* it seems possible that their cures were the result of contamination of their compound with nicotinamide.

Methods for the estimation of N′-methylnicotinamide in the urine have been developed in a number of laboratories. Most of them are basically similar in that they use a preliminary purification of the urine by adsorption on a suitable agent (zeolite) and elution with strong potassium chloride solution. The eluate is then made alkaline, extracted with butanol, and the fluorescence of the extract measured. This principle has been developed from its earlier applications to more satisfactory modifications by Najjar (*Bull. Johns Hopkins Hosp.* **74,** *392* (*1944*)), Huff and Perlzweig (*J. Biol. Chem.* **150,** *483* (*1943*)), Coulson, Ellinger, and Holden (*Biochem. J.* **38,** *150* (*1944*)), and Hochberg, Melnick, and Oser (*J. Biol. Chem.* **158,** *265* (*1945*)). A simplified method, dispensing with adsorption procedures and depending upon measurement of the heightened fluorescence of the acetone addition compound of N′-methylnicotinamide has been advanced by Huff, Perlzweig, and Tilden (*Federation Proceedings* **4,** *92* (*1945*)). When comparing the work of different authors it is necessary to note whether they express the results of their analyses in terms of N′-methylnicotinamide, or its chloride, or the equivalent amount of nicotinamide.

The application of these methods has produced results which cannot yet be fully evaluated, as they present contradictions which are still unresolved. That the excretion of N′-methylnicotinamide is decreased in pellagra has been reported by Roberts and Najjar (*Bull. Johns Hopkins Hosp.* **74,** *400* (*1944*)) as well as by Ellinger, Benesch, and Kay (*Lancet* **I,** *432* (*1945*)), and that the amount excreted after an oral dose of nicotinamide is proportional to the nutritional well-being of the individual has been observed by Ruffin, Cayer, and Perlzweig (*Gastroenterology* **3,** *340* (*1944*); *South. Med. J.* **38,** *111* (*1945*)). However, the latter workers could not correlate the excretion levels with clinical signs of the plane of nicotinic acid metabolism any better than with the general state of nutrition, and the difference between the mean excretions of various groups was not significant in view of the wide variation in each group. In contrast with these results, Mickelsen and Erickson (*Proc. Soc. Exp. Biol. Med.* **58,** *33* (*1945*)) reported that the level of excretion of N′-methylnicotinamide is independent of the intake of nicotinic acid and amide in the diet within the range 0.12 to 22.4 mg. daily. Also Ellinger, Benesch, and Kay (*loc. cit.*) observed that oral doses of succinylsulfathiazole caused the urinary excretion of N′-methylnicotinamide to decrease, and concluded that this effect was due to suppression of the synthesis of nicotinamide by the intestinal flora, which flora they believed normally supplies a considerable part of the need of man for this vitamin.

Another unsolved question is the fate of the greater part of the ingested nicotinamide.

After an oral dose of nicotinamide, no more than one-fifth or one quarter of the amount ingested can be accounted for in the form of known pyridine derivatives (Sarett, Huff, and Perlzweig, *J. Nutrition* **23,** *23* (*1942*); Ellinger and Coulson, *Biochem. J.* **38,** *265* (*1944*); Hochberg, Melnick, and Oser, *J. Biol. Chem.* **158,** *265* (*1945*)). On a good diet containing 23 mg. of nicotinic acid (chiefly as the amide) the last mentioned authors found a daily excretion of 3 to 8 mg. N′-methylnicotinamide (expressed as nicotinamide), the average being 24 per cent of the intake. Huff (*Thesis, Duke University* (*1945*)) has shown that if an oral dose of N′-methylnicotinamide is taken, just about the same amount will appear in the urine as if the equivalent dose of nicotinamide were ingested. It thus appears that the pathway of degradation of nicotinamide leads through N′-methylnicotinamide, which is largely destroyed by man. The dog presents a striking species contrast, for it was shown by Sarett (*J. Nutrition* **23,** *35* (*1942*)) that when on a good diet, dogs will excrete the whole of a 100 mg. dose of nicotinamide as a mixture of "trigonelline" and nicotinuric acid.

NUTRITION IN PREGNANCY

Pregnancy offers to the nutritionist a challenge and, at the same time, presents questions which are difficult to answer. The result is that a number of statements and claims have been made regarding nutritional requirements in pregnancy which are not easily supported by facts. Lund (*J. Am. Med. Assn.* **128,** *344* (*1945*)), in discussing the problem of nutrition in pregnancy, makes the important point that in the majority of studies attempting to determine the dietary requirements during pregnancy, the capacity for storage has not been taken into account. He points out that the pregnant woman stores many substances to a degree beyond her requirements and that of the fetus. This creates a reserve for emergencies. If these emergencies fail to develop the reserves may be excreted during the puerperium. Water is one exampie of this, as are nitrogen, calcium (Macy and Hunscher, *Am. J. Obst. Gynec.* **27,** *878* (*1934*)), and vitamin A. Iron is retained by women during pregnancy (Coons, *J. Biol. Chem.* **97,** *215* (*1932*)). The obstetrician, therefore, must not treat his patient according to the nutritional standards of any other persons or groups of persons but as an individual problem. The fact that comparatively little is actually known with regard to the nutritional requirements in a physiologic state which undoubtedly produces a nutritional strain, has provided an unusual opportunity for food faddists.

Lund has found that the vitamin A plasma level tends to decrease during the course of pregnancy. This occurs whether the initial level is comparatively high or is at a lower range. The decrease occurs most prominently in the third trimester of pregnancy and in all instances was observed to be corrected postpartum by a rapid elevation of the plasma vitamin A content. The average increase was roughly 33 per cent. The gestational decrease in plasma vitamin A did not seem to be due to fetal demands alone for it occurred when 25,000 I.U. of vitamin A were taken daily, an obviously excessive amount. Neither did it appear to be associated with the hydremia of pregnancy since the decrease was sometimes most pronounced during the last few weeks of pregnancy, whereas hydremia normally diminishes at that time. Concomitant determination of plasma carotene failed to show changes similar to those in vitamin A. Once elevated, plasma values were readily maintained at "normal" levels during lactation.

This investigator's study of the plasma ascorbic acid content of 197 pregnant women revealed a wide variation which, in general, reflected their dietary intake. As might be expected, a marked seasonal variation was noted.

The nutrition of the fetus is a subject of great interest. All evidence points to the fact that the fetus is more or less a parasite and takes what nutriments it needs, sometimes at the mother's expense. The weight of the fetus, for example, cannot be influenced by maternal dietary restrictions, short of starvation. It must be kept in mind, however, that the fetus cannot obtain something that the mother does not have. Thus, Burke, Beal, Kirkwood, and Stuart (*Am. J. Obst. Gynec.* **46,** *38* (*1943*)) observed a high incidence of physically unfit infants born of mothers with poor and very poor food intake. It is well recognized (Strauss, *J. Clin. Invest.* **12,** *345* (*1933*)) that a maternal deficiency of iron leads to poor fetal storage and though the infant at birth has normal hemoglobin values it may develop anemia of marked degree during the first year of life.

The vitamin A values of the newborn infant are low, the range being about one-half those of the adult. The fetal values according to Lund's studies are completely independent of maternal values and cannot be elevated even when the mother is given enormous doses (330,000 I.U.) of vitamin A daily before delivery. Carotene, on the other hand, apparently passes the placenta regularly since fetal values, although only one-tenth as high as those in the adult, were usually directly related to those of the mother. This investigator found that fetal values for ascorbic acid were always higher than maternal values but, in general, the two were roughly correlated. It was most unusual to find values in the newborn infant in the "deficiency" range. The lowest value recorded was 0.10 mg. per 100 ml., in an instance where the maternal value was 0.0. The plasma ascorbic acid values in 5 newborn infants were between 0.25 and 0.60 mg., in 17 instances they fell between 0.61 and 1.00 mg., and in the remaining 71 infants the values were still higher.

The general tenor of this report is sound and objective. It is strange to find, therefore, the conclusion drawn that "at least some, if not all of the retinal hemorrhages seen in hyperemesis gravidarum are due to increased capillary fragility induced by lack of ascorbic acid," for this conclusion is based on observations in only 3 cases. In these, following administration of large doses of ascorbic acid parenterally no further hemorrhages were observed. No information is given concerning the ascorbic acid status of these 3 individuals prior to treatment.

PELLAGRA AND CORN

Because endemic pellagra has appeared nearly exclusively among populations which use corn as a staple cereal, the hypothesis that corn plays a positive role in the etiology of pellagra, known as the zeistic hypothesis, has commanded continued attention. Pellagra appears to have been recognized in Europe only after the introduction of corn from the Americas, although in this country only occasional cases of pellagra were reported before the present century in spite of the staple use of corn since the days of the early colonists. An early variation of the zeistic hypothesis was that pellagra was caused by a toxic action of spoiled corn, but no conclusive evidence was ever attained to support this belief.

In 1912 Funk (*J. State Med.* **20,** *341* (*1912*)) coined the term "vitamine" and made the suggestion that pellagra is caused by a vitamine deficiency. The investigations of Goldberger and his associates of the

U. S. Public Health Service established beyond doubt that pellagra can be prevented and even cured by the addition of selected foods to the diet, and that therefore this disease was caused by a nutritional deficiency. The particular food factor responsible was variously termed the P-P factor and vitamin B_2, and the general view of the nutritional origin of pellagra has steadily gained ground from 1926 onward. Following the discovery in 1937 of the function of nicotinic acid as a pellagra-preventive vitamin, there has been a general tendency to regard a deficiency of nicotinic acid as the sole important causative agent of pellagra.

Nevertheless, there remained a number of observations which were difficult to reconcile with this view. It appeared from the work of Aykroyd (*Biochem. J.* **24,** *1479 (1930)*) that corn contains more "vitamin B_2" than rice or millet, although rice and millet eaters have never suffered endemic pellagra as have corn eaters. In calculating the nicotinic acid content of the diet of two population groups, Aykroyd and Swaminathan (*Indian J. Med. Res.* **27,** *667 (1940)*) found that the staple rice diet of Southern India afforded approximately 5 mg. of nicotinic acid daily and pellagra was rare, while the staple corn diet of Moldavia supplied 15 mg. but pellagra was endemic. Similarly it was calculated by Dann (*Federation Proceedings* **3,** *159 (1944)*) that the average intake of nicotinic acid in Wayne County, N. C. rural sections in 1942 (when corn was a very minor element of the diet and pellagra was not encountered there) was 7.2 mg. daily; exactly the same figure was obtained on calculation of the nicotinic acid intake of the experimental subjects of Goldberger and Wheeler (*U.S.P.H.S. Hygienic Lab. Bull.* **120,** *7 (1920)*) in whom pellagra was induced by feeding a diet containing a high proportion of corn. These calculations suggest that when the proportion of corn in the diet is increased, a larger amount of nicotinic acid is required to prevent the appearance of pellagra; in other words, that there is an apparent antagonism between nicotinic acid and corn.

Laboratory work on blacktongue, the canine disease analogous to human pellagra, has also furnished new evidence bearing on this problem. Handler and Dann (*J. Biol. Chem.* **145,** *145 (1942)*) reported that dogs which developed blacktongue when kept on a diet containing corn meal could often be cured by treatment with saline, and live to become demonstrably much more severely nicotinic acid deficient, without relapsing into blacktongue. Handler (*Proc. Soc. Exp. Biol. Med.* **52,** *263 (1943)*) observed that dogs maintained on a simplified ration lower in nicotinic acid than the Goldberger type of ration containing corn meal developed blacktongue much less rapidly and less regularly, and was also led to consider the possibility that corn itself plays a positive part in the etiology of blacktongue and pellagra.

The evidence of antagonism between corn and nicotinic acid has recently been significantly extended by the observations of Krehl, Teply, and Elvehjem (*Science* **101,** *283 (1945)*), who demonstrated that when 2 parts by weight of corn meal or corn grits were added to 3 parts of a simplified low protein diet, the growth of rats on the diet was greatly reduced. When corn grits were added, growth was almost prevented, but the addition of 1 mg. nicotinic acid per 100 g. diet restored growth to the level observed when the diet contained no corn products. Thus a growth inhibition, attributable to nicotinic acid deficiency, was produced by corn in a species which does not suffer from such a deficiency when given a simplified diet devoid of nicotinic acid. Indeed it is known that on such a diet the growing rat lays down new tissue containing a normal amount of nicotinic acid. Krehl, Teply, and Elvehjem also observed that if the protein (casein) of the basal diet was raised from 15 to 20 per cent, the growth inhibition could no longer be produced by adding corn to the diet.

These experiments have provided the

most direct and convincing evidence yet adduced to show that corn plays a definite part in counteracting nicotinic acid, and therefore in causing pellagra. In a second report on experiments with the rat Krehl, Teply, Sarma, and Elvehjem (*Science* **101,** *489 (1945)*) have presented evidence which suggests how the corn acts. Following up the finding that more casein in the basal diet prevented the inhibiting effect of corn, they examined the effect of adding lysine and tryptophane, since these two amino acids are well supplied by casein but poorly supplied by corn. Lysine did not exert any action, but 0.05 per cent of 1(-)tryptophane prevented growth inhibition by the corn products as effectively as did the nicotinic acid. The interchangeability of the tryptophane and nicotinic acid in this phenomenon is not yet fully explained, but the authors consider (on the basis of further evidence not yet published) that it may be accounted for by changes in the intestinal flora. Presumably this would mean that the diets containing corn products provide intestinal contents no longer capable of supporting the growth of a sufficient number of organisms capable of synthesizing nicotinic acid and supplying it to the rat, thus leading to a nicotinic acid deficiency. The deficiency can then be prevented directly by adding nicotinic acid to the diet, or indirectly by adding tryptophane, which would promote the growth of the nicotinic acid producing organisms.

It seems plausible to expect that similar changes may occur in the human intestinal flora as a result of including a high proportion of corn in the diet, which would modify the dietary requirement for nicotinic acid, and would account for the appearance of endemic pellagra among corn eaters only. These newest studies on the rat do however discount the hypothesis that corn acts to induce pellagra because of a toxin which it contains; it now appears that its action may be traced to a dual deficiency—of both nicotinic acid and tryptophane. Krehl, Teply, Sarma, and Elvehjem observed that addition of an equal amount of polished rice instead of corn grits to the diet caused no growth restriction; although rice contains less nicotinic acid than corn, they point out that it contains more tryptophane. Thus their observations would reasonably account for the appearance of pellagra on corn diets (as in Moldavia) and its absence among rice eaters who, as in Southern India, ingest less nicotinic acid than the corn eaters.

EFFECT OF PYRIDOXINE AND PANTOTHENIC ACID DEFICIENCIES ON THE NERVOUS TISSUES OF SWINE

In 1938 Wintrobe, Mitchell, and Kolb (*J. Exp. Med.* **68,** *207 (1938)*) described changes in the nervous system of swine which they attributed to a dietary deficiency. In this study it was noted that after a certain length of time, swine receiving a diet consisting of casein, sugar, lard, cod liver oil, minerals, thiamine, and riboflavin, developed striking incoordination in gait and eventual paralysis. Histologic examination of the nervous system revealed marked degenerative changes in the peripheral nerves, in the dorsal roots, in the ganglion cells, and in the posterior column of the spinal cord. This they described as a sensory neuron degeneration. The degeneration involved axis cylinders as well as myelin sheaths.

As newer members of the vitamin B group became available in crystalline form, it was possible by the addition of niacin, pyridoxine, choline, and pantothenic acid to the diet, to secure good growth in pigs without symptoms or signs of changes in the nervous system. The latter was found to be intact even on histologic examination (Wintrobe *et al.*, *J. Nutrition* **24,** *345 (1942)*).

In later studies it was possible for these workers to show that an abnormal gait as well as changes in the nervous system developed when the diet was lacking only in pyridoxine or in pantothenic acid.

In the most recent report by these workers (Follis and Wintrobe, *J. Exp. Med.* **81,** *539 (1945)*) the results of an investigation of the nature of the changes in the nervous system produced by pyridoxine and by pantothenic acid deficiency are described. One group of animals was given a diet complete so far as is known at this time except for pyridoxine, another group received a similar diet containing pyridoxine but with pantothenic acid omitted, and a third group received a diet in which both of these vitamins were lacking. The animals were observed carefully during life and were sacrificed at various periods during the development of the deficiency state.

The animals lacking pyridoxine showed some impairment in growth, developed a severe anemia associated with a rise in plasma iron concentration and marked hemosiderosis of the spleen, liver, and bone marrow, epileptiform seizures, fatty infiltration of the liver, and marked abnormalities in gait accompanied by lesions in the nervous system. The ataxia first manifested itself as a slightly high lift of the hind legs together with a swaying of the hindquarters in walking. Often the hind legs twisted in one direction or another. In walking the base became broad, or the legs folded under with the result that the pig stumbled or fell frequently. Eventually fore and hind legs became involved and in severe cases the animal was completely incapacitated.

After nine or ten weeks on such a deficient regimen, and after suspicious physiologic disturbances had been observed, distinct histologic changes could be demonstrated in the nervous system. Demyelinization of the peripheral nerves (brachial and sciatic) was the earliest morphologic change noted. The nerve fibers of larger diameters seemed to be the most affected. The degeneration was characterized by the appearance of small droplets of neutral fat and by the loss of the fine reticular structure of the myelin sheath in the Weigert preparations. Axis cylinder stains showed questionable degeneration of these structures in the earliest period of the deficiency. Later, definite changes were observed in the axis cylinders. Myelin degeneration was found in the early stages only in the peripheral nerves, but in later stages was also observed in the dorsal roots. Necrotic cells were found in the dorsal root ganglia of those animals showing the most marked changes, as well as foci where cells had already undergone phagocytosis.

Pantothenic acid deficiency in swine was observed to be associated with a very striking disturbance in growth, loss of hair, cough, and excessive nasal secretion, changes in the tongue, diarrhea associated with an ulcerative colitis, and ataxia accompanied by lesions of the sensory neurons. In these animals, the abnormality in gait consisted in a sudden lifting of one of the limbs from the ground as if it were painful. The gait revealed a broadening base and a jerky, almost military goose step appeared. The twisting of the legs seen in pyridoxine deficiency was usually absent. Eventually the pig was unable to stand or walk. Morphologic changes were observed in animals even though no signs of ataxia had been discovered during life. Thus, unlike the findings in pyridoxine deficiency, it would appear that pathologic changes may precede any objective evidence of abnormal gait resulting from pantothenic acid deficiency. The earliest morphologic change was found in the dorsal root ganglion cells. These cells exhibited classic signs of chromatolysis. The Nissl bodies became finer and eventually dissolved. This process in most instances began in the center of the cell and proceeded peripherally. During the early stages of the deficiency, when changes were observed in the ganglion cells, no alterations in the peripheral nerves or dorsal roots could be demonstrated. Later, however, loss of

myelin was found in the peripheral nerves of all animals. Axis cylinder degeneration was present as well. In a few animals chromatolytic cells were present in the anterior horns of the spinal cord also. In animals deficient both in pyridoxine and pantothenic acid the lesions found were of the same morphologic character as those observed when either of these vitamins was lacking, and clearly represented a combination of the two types of deficiencies.

These observations explain the earlier findings of these workers and show the progress of the lesion produced by pyridoxine and pantothenic acid deficiency in swine. The histologic picture produced by a prolonged deficiency of either of these vitamins is the same, but the changes begin in a different location. Apparently, when pyridoxine is lacking, myelin degeneration of the peripheral portions of the sensory nerve and axis cylinder degeneration are the earliest changes. In such animals alterations in the dorsal root ganglion cells were a late development. In contrast, in animals deficient in pantothenic acid, pronounced chromatolysis of the dorsal root ganglion cells was the first change. Only subsequently did changes in the peripheral nerves take place. In both groups changes in the posterior columns of the spinal cord were a late event. It would appear that the metabolic changes induced by deficiencies of pyridoxine and of pantothenic acid being different, the initial damage is also different.

These findings are consistent with observations on diseases of the nervous system in man. These have been classed as being of two types, namely, myelinoclastic and polioclastic. In the first group myelin degeneration is the first manifestation of the disease and is followed later by axonal and cellular degeneration. Multiple sclerosis, Schilder's disease, and the vaccinial type of encephalitis are myelinoclastic diseases. In the second group the first discernible changes are found in the neuron itself; axonal and myelin degeneration follow. Poliomyelitis, rabies, epidemic encephalitis, herpes simplex in rabbits, and damage resulting from anoxia are examples of the polioclastic diseases. According to this classification pyridoxine deficiency would fall into the myelinoclastic group while the lesions occurring in pantothenic acid deficient swine would be classed with the polioclastic diseases.

An interesting question is the possible relation of the experimental deficiencies observed in swine to similar disorders in man. Another matter of great importance is the manner in which these vitamin deficiencies lead to degenerative changes in the nervous system. It would be of interest to carry out studies on the metabolism of dorsal root ganglion cells in relation to that of cells from other portions of the nervous system. The ultraviolet microscopic technic may be helpful. Already, Gersh and Bodian (*J. Cell. Comp. Physiol.* **21,** *253 (1943)*) have shown that large dorsal ganglion cells contain greater amounts of nucleotides than anterior horn cells. This finding may be of significance in explaining the pathogenesis of the neurologic changes in swine deficient in pantothenic acid.

NUTRITIVE VALUE OF THE FATTY ACIDS OF BUTTER

Many studies have been made in which the nutritive values of natural fats or commercial food fats have been compared, but only a few studies have been recorded of the relative nutritive value of different fatty acid fractions derived from a single natural fat. Some years ago Boutwell, Geyer, Elvehjem, and Hart (*J. Dairy Sci.* **24,** *1027 (1941)*) obtained evidence that the saturated fatty acids of butter produced better growth of young rats than did the unsaturated acids of the butter or the butterfat itself. This sub-

ject has now been pursued further by Brown and Bloor (*J. Nutrition* **29,** *349 (1945)*), in a well-designed series of experiments.

Brown and Bloor fractionated the acids of butterfat into five fractions. The fat was first saponified, acidified and steam distilled, to separate the volatile acids which were titrated and estimated as butyric acid. The residue was extracted with distilled ether and the acids so extracted were separated by use of the lead salts into "solid" and "liquid" acids. Each of these preparations was then separated by fractional distillation of the methyl esters into two fractions having average molecular weights as different as possible. The average titration equivalents and iodine values for the high molecular liquid acids were 279 and 98 respectively; low molecular liquid acids, 226 and 35; high molecular solid acids, 271 and 7.8; and low molecular solid acids, 248 and 0.6.

The diet in which these fatty acids was incorporated for testing contained 66.6 per cent whole wheat flour, 23.3 per cent of skim milk powder, and 10 per cent of the test acids, with an addition of sodium chloride to the extent of 2 per cent of the weight of the flour. Each of the fatty acid fractions was tested, together with whole butterfat. In addition to the test diet fed ad libitum each rat was given daily 3 g. of raw carrot and a drop of a solution of vitamin D in propylene glycol. The complete feeding experiment was performed three times, using 24 rats each time. Change in weight and food consumption were measured in each experiment, and in the third experiment the feces were collected throughout the last two weeks. At the end of the feeding period the rats were autopsied and examined for gross abnormalities, and the livers were removed for analysis. Both vitamin A and the lipids were measured in the livers, and the feces were also analyzed for lipids.

The efficiency of the diets containing the different acids tested for growth was expressed as the average ratio for each group obtained by dividing the gain in weight by the food consumption of the individual rat. The results of the three experiments showed some variation, but certain regularities encountered did show that the butter and liquid acid diets were superior to the solid acid and volatile acid diets. This is regarded as due to a poorer absorption of the solid acids from the alimentary tract, which was demonstrated by the greater excretion of fatty acids by animals receiving the solid acids. Such an explanation would not cover the volatile acids, which were least efficient of all the fractions tested. The percentage absorption of the different fats and acids, expressed as an average for all rats in all experiments, was for butter 93, volatile acids 91, low liquid acids 95, high liquid acids 93, low solid acids 71, and high solid acids 42.

The vitamin A measurements on the liver tissues also showed a marked difference in the ability of the rats in different groups to store vitamin A ingested as the carotene of raw carrots. Here again the efficiency of the various fractions of fatty acids in producing absorption of the carotene and its storage as vitamin A varied, and again the liquid (unsaturated) acids were more efficient than the solid (saturated) acids and the volatile acids were least efficient of all. Twice as much vitamin A was stored on the liquid acid as on the solid acid diets. The liver lipids were not found to vary markedly in quantity or in degree of unsaturation from one group to another. On the other hand the degree of unsaturation of the fecal lipids was much less when the solid acids were fed.

Thus the work of Brown and Bloor appears to establish that the unsaturated fatty acids of butter are superior to the saturated acids both for growth and for storage of vitamin A. Indeed both the low and high molecular unsaturated acids were as efficient for growth as butterfat itself. This result appears to contradict the finding of Boutwell, Geyer, Elvehjem, and Hart (*loc. cit.*) but the different basal diets employed

by the two groups of workers will probably account for the different conclusions drawn. Brown and Bloor used a basal diet which contained only the fat of the wheat flour, but the earlier workers used corn oil in their diet in addition to the test fat. Under these conditions the efficiency being measured was that of the mixture of the butterfat fractions plus the corn oil, and such mixtures may well differ from the butter fractions above.

NUTRITIONAL SURVEY OF PATIENTS WITH SKIN DISORDERS

Taking into consideration that signs of dietary deficiency are frequently found in the skin, Callaway, Milam, and Noojin (*Arch. Dermat. Syph.* **51,** *266 (1945)*) undertook a study of 354 patients seen in the skin clinic of the Duke Hospital in Durham, North Carolina. In addition to the usual history and physical examination, dietary data were obtained by means of a questionnaire filled in for each patient by the staff of the clinic, and blood examinations were made for ascorbic acid, vitamin A, carotene, hemoglobin, red cell count, and volume of packed red cells. The patients included 303 white persons and 51 Negroes. Among the 354 cases, 71 different types of skin disorder were observed. These included syphilis, contact dermatitis, neurodermatosis, dermatophytosis, psoriasis, and pyoderma.

The mean plasma ascorbic acid, vitamin A, and carotene levels in these cases corresponded to those found in field surveys in North Carolina in persons without skin disorder. In general the frequency of low levels of vitamin in the plasma or of high levels was also similar. More consistently, low levels of ascorbic acid were found, however, in patients with urticaria (hives), lupus erythematosus disseminatus, and pyoderma. Exceptionally high values were observed in patients with common acne. The lowest levels for vitamin A were found in dermatitis due to medicaments and in pyoderma. In the patients with psoriasis relatively high vitamin A levels and low carotene levels were noted. The significance of these observations is not apparent. In patients with ichthyosis, a condition which has been considered by some workers as caused by a deficiency of vitamin A, normal levels of plasma vitamin A were observed.

It was clear from these studies that in general the nutritional status of the patients in the skin clinic closely paralleled that of inhabitants of corresponding rural communities as studied by one of the investigators (Milam and Anderson, *South. Med. J.* **37,** *597 (1944)*). The nutritional status of the 354 patients appeared to be representative of the milieu from which they came and no special nutritional deficiency could be singled out as a factor in their dermatologic lesions. Within the limitations of these studies one finds no justification for the indiscriminate use of vitamins for the treatment of skin disorders.

"FOLIC ACID" IN MILK

Evidence that there exists in milk a form of "folic acid" which is inactive in the microbiologic test but active for the rat has been presented in detail by Wright and co-workers (*J. Nutrition* **29,** *289 (1945)*). Welch and Wright (*Science* **100,** *153 (1944)*) had previously postulated the existence of a microbiologically inactive "folic acid"-like material which possessed vitamin activity for the rat, when they found that the incorporation of succinylsulfathiazole up to 10 per cent of a ration of powdered whole milk did not produce "folic acid" deficiency in the rat such as is induced by adding 2 per

cent of succinylsulfathiazole to purified diets. This was taken as evidence that the milk, which on the basis of microbiologic assay does not contain significantly more "folic acid" than the purified diets, contains a component which can be used by the rat in place of the microbiologically active material. Binkley *et al.* (*Science* **100,** *36* (*1944*)) independently produced evidence for the existence of a combined form of "folic acid" (or vitamin Bc) by demonstrating that yeast extract contains a substance available to the chick but not to Lactobacillus casei, and that this material could be made available to the microorganism by submitting the yeast extract to enzymic digestion. Mims, Totter, and Day (*J. Biol. Chem.* **155,** *401* (*1944*)) have developed the assay of such combined "folic acid," while Mallory, Mims, Totter, and Day (*Ibid.* **156,** *317* (*1944*)) have demonstrated that the growth-promoting and leucocytopoietic effects of yeast and liver in the rat are proportional to the total amount of "folic acid" measured microbiologically after enzymic digestion, rather than to the amount measured microbiologically before such digestion.

Wright *et al.* (*loc. cit.*) have made further studies of the effects of adding succinylsulfathiazole to a diet of powdered whole milk. When 1, 2, or 5 per cent of the drug was added to the powdered milk, no effect on the growth rate of rats through a period of fourteen weeks was noted. With 10 per cent of the drug, one rat out of 11 died, and the remainder grew slightly more slowly; with 20 per cent, 4 rats out of 8 died within eight weeks, and the remainder grew very poorly. The "folic acid" content (determined microbiologically) of the livers of the rats was studied, since Wright and Welch (*J. Nutrition* **27,** *55* (*1944*)) have shown that a deficiency of "folic acid" in the rat is accompanied by a low "folic acid" content in the liver tissue. Although with rats on purified diets, "folic acid" deficiency could be produced by the addition of 2 or 5 per cent of succinylsulfathiazole to the diet, and was accompanied by low "folic acid" levels in the liver, it was necessary to add 20 per cent to powdered whole milk in order to produce a liver "folic acid" level below 0.5 microgram per gram, the level generally found in "folic acid" deficiency.

The fecal excretion of the rats receiving powdered whole milk with various levels of the drug was also examined, and here it was noted that the deficient animals still excreted a considerable amount of "folic acid," more for instance than healthy rats on a purified diet not containing succinylsulfathiazole. At the end of the experiment, total and differential leucocyte counts on the blood of the rats were made, and these confirmed the results already described in that no blood dyscrasia was found in the rats which received less than 10 per cent of succinylsulfathiazole in the powdered milk. From the sum of these observations the authors conclude that powdered milk contains significant quantities of "potential folic acid" which is unavailable to microorganisms but is available to the rat.

ADAPTATION TO FASTING

An outstanding attribute of animals in general, and of man in particular, is the ability to adapt to stress conditions. Adaptation may be defined as an alteration in the organism consequent to the application of a noxious agent or condition of stress which enhances the ability of the organism to withstand such agent or stress. It is well known that acclimatization to high altitudes or hot climates can occur, and that specific adaptative mechanisms are involved. Mitchell (*J. Am. Dietet. Assn.* **20,** *511* (*1944*)) has stressed the ability of the animal organism to survive periods of marked re-

striction in various specific nutrients. Such survival is no doubt to be explained in part on the basis of adaptation.

Folin and Denis (*J. Biol. Chem.* **21,** *183 (1915)*) observed that adaptation to fasting occurs in obese individuals, in that the amounts of urinary nitrogen and ketones decrease with successive fasts. Taylor *et al.* (*Am. J. Physiol.* **143,** *148 (1945)*) have now reported that the ability of man to do hard work during fasting is increased after successive fasts, and have investigated some of the biochemical responses concerned in this adaptation. Four young men underwent five successive fasts at intervals of five or six weeks. For three weeks prior to each fast, the diet was carefully regulated. During the entire interim periods standard amounts of work were performed. This work was increased just before the fast until it equaled the amount of work to be performed in the fasting period. During the two and a half day fasting period itself a total energy deficit of about 10,000 calories was induced, and a number of biochemical and psychomotor tests were performed. The manual work consisted of about ten hours (four hours per day) spent on a treadmill with an average expenditure of 550 calories per hour. At the outset of each fast the men were apparently in nearly the same physical condition, as evidenced by the average working pulse rate, and the blood lactate values obtained after a fixed period of anaerobic work. There was, however, indication of improvement on training in the performance of certain of the psychomotor tests from comparison of the control periods before and after the first fast with the control periods for the fifth fast.

Only data for the fifth and first fasts are presented. During the second and third days of the fifth fast, in comparison with the corresponding portion of the first fast, the working blood sugar values averaged 13 mg. per cent higher, the resting blood acetone levels 20 per cent lower, and the working ventilation rates were about 20 per cent lower. Thus on these three scores, adaptation had occurred. On the other hand, the working pulse rates and respiratory quotients were nearly identical. During the second twenty-four hours of the fifth fast, 40 per cent less acetone and 8 per cent more nitrogen were excreted than during the same period of the first fast. The results of the psychomotor tests are somewhat equivocal. There was less absolute deterioration in the performance of these tests during the fifth fast; however, since the tests were performed better during the control periods of the fifth fast, the percentage deterioration for the first and fifth fasting periods was about the same. There was no correlation between the blood sugar levels and the psychomotor performance. Theoretically, increased gluconeogenesis from protein might account for the higher blood sugar and lower blood ketone levels after adaptation. There was, however, no correlation between blood sugar or ketone with nitrogen excretion. The observed increase in urinary nitrogen of 0.8 g. per day would correspond to a maximum increased gluconeogenesis of only about 3 g. of glucose per day, a trifling amount.

It thus appears that fasting may be added to the imposing list of stress conditions toward which adaptation can occur. The authors point out that the following obscurities remain to be elucidated: (1) the complete mechanism of adaptation to fasting; (2) the number of fasts required to produce such adaptation; and (3) the time period over which the adaptive effect will persist.

THE VITAMIN B-COMPLEX AND COFFEE

Because of the almost universal use of coffee as a beverage in the United States, contributions to our knowledge of its food value are of considerable importance. The

complex organic composition of roasted coffee may be indicated by the following data from the compilation of Winton and Winton ("The Structure and Composition of Foods," *Vol. IV, John Wiley and Sons, Inc., New York (1939)*): Protein, 11.23 to 15.74 per cent; fat, 13.13 to 16.51 per cent; sugar, 0.41 to 2.45 per cent; caffeine, 0.82 to 2.19 per cent; choline, 0.022 per cent; trigonelline, 0.228 to 0.245 per cent. In addition, coffee is known to contain pyrrol, pyridine, furfural, amyl alcohol, a variety of fatty acids, phenols, the alkaloid coffearine, coffalic acid, and chlorogenic acid. This latter compound may be hydrolyzed to yield quinic acid (1,3,4,5-tetrahydroxy-hexahydrobenzoic acid) and caffeic acid (beta-3,4-dihydroxyphenyl-acrylic acid). A number of other compounds have been identified in coffee.

Little has been published regarding the vitamin content of coffee, but since it contains trigonelline it would not be surprising if nicotinic acid or other pyridine derivatives were also present. Teply, Krehl, and Elvehjem (*Arch. Biochem.* **6,** *139 (1945)*) have investigated the niacin content of several coffees and report a number of interesting observations. First, microbiologic assays with Lactobacillus arabinosis of roasted coffee have indicated a niacin content of 8.0 to 10.9 mg. per 100 grams. By a similar method it was found that the niacin activity of coffee beverage ranged from 0.78 to 1.28 mg. per cup. The concentration of this active substance was less in decaffeinated coffee and was only one-tenth as great in an extract of green coffee. Furthermore, a material in green coffee could be activated by treatment with moderately strong alkali.

In order to test for the availability of the microbiologically active material an extract of roasted coffee was given to chicks maintained on a diet low in nicotinic acid (Briggs, Mills, Elvehjem, and Hart, *Proc. Soc. Exp. Biol. Med.* **51,** *59 (1942)*). Poor and erratic growth resulted with the aqueous extract, and a more purified preparation was found to be only one-fifth as active for the chick as for L. arabinosus. The interpretation of these results is difficult because the preparations probably contained materials toxic to the chick.

A norit eluate of coffee was fed to niacin deficient dogs in an effort to determine the niacin content. No activity was observed in the 3 dogs tested, but the same 3 dogs later failed to respond to nicotinic acid. However, after failing to respond to the coffee preparation and to niacin, they made weight recoveries when given biotin (route not stated). In a single experiment in which the basal ration was supplemented with inositol, p-aminobenzoic acid, and biotin, the response of the dog to a coffee concentrate indicated approximately the same activity as did the microbiologic assay. The evidence is insufficient, however, to establish the true niacin content of coffee.

When roasted coffee was added, along with niacin, to the basal diet the dogs showed a variety of signs: diuresis, catharsis, weight loss, flaccid paralysis, graying of the hair, a "weepy eye" condition, and convulsions. The paralysis appeared to be relieved by inositol, the eye condition by biotin. The occurrence of this paralysis, which was relieved by a water soluble factor, recalls the paralysis which Smith (*Science* **100,** *389 (1944)*) reported as occurring in dogs maintained on a purified diet and which was cured by administration of biotin.

The incorporation of finely ground roasted coffee into a purified diet which was fed to rats also led to the development of symptoms: viz., rapidly developing alopecia, weight loss, edema, and death. Inositol and whole liver did not entirely counteract these signs. The feeding of quinic acid, structurally related to inositol (see above), did not produce a growth depression or alopecia. The feeding of crystalline caffeine to rats did result in an alopecia, growth depression, and, at 1 to 2 per cent levels in the diet, edema and death. Decaffeinated coffee produced a depression of growth but no alopecia.

The results suggest that the inclusion of very large amounts of coffee in the diet may produce a biotin and an inositol deficiency in the dog and decreased growth in rats. The alopecia produced in rats would seem to be related to the intake of caffeine. The amount of coffee fed in these studies is much greater than that usually consumed in human dietaries. The data are not conclusive as to the availability for animals of the nicotinic acid apparently present in coffee as measured by microbiologic tests.

THE EFFECT OF VITAMINS A AND D ON BONE FRACTURE HEALING

By the use of radioactive strontium Copp and Greenberg (*J. Nutrition* **29**, *261* (*1945*)) have made a valuable quantitative study of the healing of bones, and the effects exerted on healing by varying levels of vitamin A and vitamin D in the diet. Working with rats, these authors produced fractures of the right fibula, choosing this bone since it is naturally splinted by the tibia so that no care of the fracture is necessary. The rats were sacrificed at four day intervals over a period up to one month following the production of fracture, and the healed fracture was examined for rate of calcification and for strength, being compared with the unfractured left fibula as a control.

In order to follow the calcification activity each rat was injected intraperitoneally twenty-four hours before it was killed, with one fifth of a milligram of labeled strontium (i. e., strontium labeled by containing some of its radioactive isotope). At autopsy the fractured fibula was removed and its content of labeled strontium determined by measuring its radiation directly with a Geiger-Müller counter; the unfractured fibula was also treated in the same way. The margin of extra labeled strontium found in the fractured bone was used as a measure of the calcification activity in the callus. That the extra calcification activity in the fractured bone occurred in the callus was verified by taking radioautographs—i.e. by laying the bone on roentgen film and permitting the radiations to darken the film and so produce an image. The darkest parts of the image were produced by the sites of greatest activity in deposition of minerals.

The strength of the bones was determined by an apparatus of the same design as that used by engineers to measure the breaking strength of a beam. Knife edges 1 cm. apart supported the bone, and downward pressure was applied to two other knife edges resting on the bone between its supports. The supports were placed on the pan of a Toledo scale, and the downward pressure until the moment of breaking was read on the indicator. In this way a figure was obtained which served as a measure of the absolute strength of the bone, and the strength was also expressed as a fraction of the strength of the unfractured bone from the same animal.

In normal young adult rats (2 to 3 months old) maintained on a stock diet, Copp and Greenberg found that the calcification activity was at a maximum from eight to sixteen days after the bone was fractured. The breaking strength of the fractured bone was negligible for the first four days, but thereafter it increased rapidly until after twelve or sixteen days it was almost as strong as the unfractured bone. For studies in vitamin A deficiency, rats were weaned at three weeks and then kept for five weeks on the U. S. P. vitamin A test diet. The bones were then fractured and the rats divided into two groups, both of which continued on the deficient diet while the rats of one group received a weekly supplement of 10,000 I. U. of vitamin A in shark liver oil; the first dose was given just after the fracture was produced. The unfractured bones of both vitamin A deficient and supplemented rats were equally as strong as the unfractured

bones of the normal rats mentioned above which were maintained on the stock diet. The fractured bones of the vitamin A-supplemented rats returned in twelve days to about the same strength as when the rats were fed stock diet, while the vitamin A-deficient rats showed a slower return to normal strength of the fractured bone. The calcification activity in both the deficient and the supplemented groups was lower than had been observed in the normal rats on stock diet, and this was correlated with the observation that the fracture callus of both groups was considerably smaller than had been observed in stock-fed rats.

Experiments with vitamin D covered both deficient and hypervitaminotic rats. All rats in the rickets experiment were fed the U. S. P. Rachitogenic Diet No. 2 from the age of 24 days, and depleted for twenty-five days, after which fractures were produced in the softened fibulas. The rats were divided into two groups, both still maintained on the same diet, but one receiving a supplement of 10 I. U. of vitamin D daily. In both groups the fractured bones reached the strength of the unfractured ones within twelve days, but this strength was only about one-quarter that of normal bone. The calcification activity among the supplemented rats was much below the activity in the fractured bones of rats on stock diet, while no calcification at all occurred in the rachitic rats. Hypervitaminosis D was induced by giving 40,000 I. U. vitamin D every four days to young adult rats fed the stock diet. In these rats the callus size and calcification activity were much less than in normal rats, and the recovery in breaking was much delayed. In addition it should be noted that the strength of the unfractured bones was less than that found in the normal rats.

The study of Copp and Greenberg provides quantitative data on the biochemical and biophysical changes which underlie the changes of bone repair previously noted by the pathologists as a result of vitamin A and vitamin D deficiency.

FAT DIGESTIBILITY IN RELATION TO GLYCERIDE STRUCTURE

A paper by Mattil and Higgins (*J. Nutrition* **29,** *255* (*1945*)) reports a well executed investigation of one aspect of the effect of structure upon the digestibility of glycerides. In general the higher melting fats are less well absorbed; nevertheless there are other important controlling factors. Thus palmitin and stearin melt at nearly the same temperature, yet it has long been known that palmitin is well digested and stearin very poorly digested, a fact which has been recently confirmed by Hoagland and Snider. These workers (*U. S. Dept. Agr. Tech. Bull.* **821** (*1942*)) failed to show any consistent relationship between melting point and digestive coefficient, and in another paper (*J. Nutrition* **26,** *219* (*1943*)) reported that tristearin and stearic acid are poorly absorbed, but tripalmitin and palmitic acid are much more completely absorbed.

This finding has now been extended by Mattil and Higgins (*loc. cit.*) with interesting results. They compared the digestibility for the rat of 4 experimental fats prepared synthetically. The diets contained 18 per cent of crude casein, 56 per cent of dextrose, and 15 per cent of the experimental fat, with salt mixture, liver extract, and yeast. The four experimental fats were, first, a mixture of 2 parts tristearin and 1 part triolein; second, a mixture of 2 parts triolein and 1 part tristearin; third, distearomono-olein; and fourth monostearodiolein. Each fat was tested on two groups of rats; the fat consumption was measured and the fecal fat was determined and corrected for fat excreted on a fat-free diet.

For each experimental fat the duplicate determinations agreed well. The mixtures of triglycerides were 39 per cent absorbed

when two-thirds was tristearin, and 69 per cent absorbed when two-thirds was triolein. For the mixed glycerides, distearomonoolein was 59 per cent absorbed and monostearodiolein was 73 per cent absorbed. Thus the oleic acid was much better absorbed than the stearic acid, and in the experimental fats containing 2 moles of stearic acid to 1 mole of oleic acid the stearic acid was absorbed much better when it was present in a mixed glyceride than when it was present as tristearin mixed with triolein. The fecal fat was analyzed; when stearic acid predominated in the experimental fat, the fecal fatty acid was about 80 per cent stearic acid and 8 to 9 per cent oleic acid, but when oleic acid predominated in the experimental fat, the fecal fatty acids were about 66 per cent stearic acid and 16 per cent oleic acid.

These results show that in a comparison of stearic and oleic acids, the acid with the lower melting point is the more digestible, but that the type of glyceride in which it occurs in the food is also an important factor in determining the digestibility of the total fat of the diet.

SULFUR BALANCE IN THE HEN

Little or nothing appears to have been known of the sulfur balance of the domestic fowl until this subject was investigated by Holman, Taylor, and Russell (*J. Nutrition* **29,** *277 (1945)*). These authors have measured the balance in thirty-five separate periods of four or twelve days each, extending over three month's time, on 4 hens. The balance periods covered times of molting, of resting without molting or laying, of low egg production, and of high egg production. Molting was defined as the period during which a hen loses more than 0.5 g. of feathers daily.

The food, feathers, excreta, and eggs were separately analyzed for sulfur, after a preliminary oxygen bomb combustion for the purpose of destroying organic matter and converting reduced sulfur to sulfate. During rest periods each of the 4 hens showed a considerable retention of sulfur; the average of twelve rest periods for the 4 hens was a retention of 44.0 mg. sulfur daily, the intake being 141 mg. and excretion 97 mg. When molting, the hens lost around 25 mg. sulfur daily in the feathers which were shed, and with smaller food consumption their intake on the average was down to 80.4 mg. and the daily loss of sulfur from the body was 0.6 mg. In periods of low egg production, on the other hand, the hens were storing sulfur, and continued on the average to do so until egg production passed the 50 per cent mark—i.e. one egg in two days. During these periods however the sulfur excretion remained high, the total loss in eggs and droppings being from 70 to 120 mg. daily, and positive balance was of course only possible because of greatly increased food consumption which yielded an intake of sulfur as high as 160 mg. daily. When egg production exceeded 50 per cent, the sulfur balance became negative in the four periods reported.

Even during these periods of high egg production the amount of sulfur excreted each day remained high, being between 90 and 100 mg., or 64 per cent of the intake, in seven periods when the production rate was 50 per cent or higher. The authors discuss briefly why such large excretions should continue when the balance is negative, but offer no evidence on this point. It is of interest to inquire, when a production rate of 58 per cent or higher produces a negative sulfur balance, how such hens maintain life while laying 300 or more eggs in consecutive seasons. One can only speculate that the breeding of heavy layers in essence is likely to be the breeding of hens which have the ability to maintain unusually high food intake, in addition to suppressing the molting partially or almost completely.

HYPOTHALAMIC LESIONS, FEEDING HABITS, AND FOOD UTILIZATION

It was observed by Long and associates (*Nutrition Reviews* **2**, *105* (*1944*)) and has now been confirmed by other investigators that damage to the hypothalamus (portion of lower brain) affects feeding habits and that these changed feeding habits may influence food utilization. These results are of interest both to the physiologist and to those engaged in nutritional research. In a paired feeding experiment it is not unusual for some of the animals to be perpetually hungry. Such animals are wont to consume their allotments of food in a very short space of time. In so far as these animals utilize their food in a different manner or with different efficiency from their paired littermates, then to that extent the purpose of the experiment is defeated.

Brobeck, Tepperman, and Long (*Yale J. Biol. Med.* **15**, *831* (*1943*)) in an investigation of hypothalamic obesity used a paired feeding technic with 12 pairs of rats. One animal in each pair had a damaged hypothalamus; the other did not. In 9 of the 12 pairs, the rats with lesions gained at the same rate as the controls. In 3 of the 12 pairs, however, the rats with lesions gained more rapidly than the controls and the authors suggested that this phenomenon may have been related to the habitually rapid eating of the rats in question. These same workers (*Ibid.* **15**, *855* (*1943*)) investigated the question further and were able to demonstrate that rats with hypothalamic lesions, or rats without lesions which were trained to eat their daily ration of food in three hours or less, showed high respiratory quotients after a test meal. One group of 20 rats without lesions was trained to eat its daily allowance of a so-called "synthetic" diet, low in fat and high in sucrose, in one hour. These animals, tested in the fifth week of training, had a mean respiratory quotient of 1.220; in this series, quotients of 1.3 were not uncommon and one rat exhibited a respiratory quotient of 1.4. Carbon dioxide production was excessive in similar animals. As pointed out by the authors, these results strongly suggest that there was an extremely rapid transformation of carbohydrate to fatty acids after glucose administration both in the trained animals without lesions and in the animals with hyperphagia due to hypothalamic damage.

Brooks, Lockwood, and Wiggins (*Federation Proceedings* **4**, *9* (*1945*)) made a study of the feeding habits of normal rats and rats with hypothalamic lesions of the type which produce obesity. Normal rats ate approximately 70 per cent of the total food consumed at night (i.e. between 6 p.m. and 6 a.m.). Meals rarely exceeded 4 g. (of mash) and averaged 1.5 to 2.0 g. The animals ate, on the average, once every three to four hours during the day and once every one to two hours at night. During the dynamic phase of hypothalamic obesity, comparable animals ate 14 to 20 g. in a single meal immediately after the container was filled with fresh food. Throughout the remaining hours they ate meals which averaged 4 to 7 g. at rather uniform intervals of once every two hours. During the static phase the eating pattern tended to revert to normal.

Brooks, Lockwood, and Wiggins studied the effect of food ingestion on the respiratory quotient of normal rats, and rats during the dynamic and static phases of hypothalamic obesity. Purina dog chow was the ration used in this study. Abnormally high quotients were found only in the dynamic phase of the obesity and a rise above unity was not obtained unless 3 g. or more, dry weight, of food were ingested at one meal. This rise above unity following a meal was not apparent immediately after the operative damage to the hypothalamus but was observable by the fourth day thereafter. It will be noted that these experiments differed from those of Tepperman *et al.* in that here

the respiratory quotients were studied following the regular meal while the Yale investigators employed test meals of glucose. Brooks conducted one experiment, however, in which test meals were used. Six animals with lesions were pair-fed with normal rats but they were given equally spaced meals never exceeding 2 g. in amount. After a test meal, the respiratory quotients of the animals with lesions rose well above unity even though periodic engorgement with food had never occurred. The exact quotients attained are not recorded in this preliminary report.

The results from the two laboratories agree in that they indicate that the feeding habits of rats are greatly altered following damage to the hypothalamus and that these alterations in feeding habits influence the manner in which food is utilized. There appears to be a further suggestion in the data presented by Brooks and co-workers that the hypothalamic injury may in itself influence food utilization.

VITAMIN A CONTENT OF HUMAN MILK AFTER SUPPLEMENTATION OF THE DIET

The effect of large vitamin A supplements on the composition of human milk has been studied by Hrubetz, Deuel, and Hanley (*J. Nutrition* **29,** *245* (*1945*)). In referring to earlier work these authors mention that McCosh *et al.* (*Ibid.* **7,** *331* (*1934*)) found no rise in the vitamin A content of the milk of 3 women after they supplemented an abundant and well chosen diet by 15 g. of cod liver oil daily. They also refer to the work of Dann (*Biochem. J.* **26,** *1072* (*1932*)) on the vitamin A content of the milk of women receiving cod liver oil supplements, but state that this supplement was given during pregnancy and continued during lactation, whereas it was actually reported given only during pregnancy.

Hrubetz, Deuel, and Hanley made measurements on the milk of 42 women who received 50,000, 100,000, or 200,000 I. U. of vitamin A daily from the sixth month of pregnancy to the end of lactation. For control figures, analyses were made of eighty-five samples of milk from normal subjects whose diets were unsupplemented, at various stages of lactation. The diet of all subjects was presumed to have been good since the subjects were under the care of an obstetrician and they came from the middle or upper economic group. The vitamin A contents of the milk samples were determined by a modification of the Carr-Price reaction. From two to ten days postpartum group 1 (no supplement), group 2 (50,000 I. U.), group 3 (100,000 I. U.), and group 4 (200,000 I. U.) subjects secreted milk with mean vitamin A contents of 331, 599, 869, and 1047 I.U. respectively per 100 ml. The mean figures for eleven to thirty days postpartum were 232, 471, 498, and 871 respectively, and for thirty-one to sixty days postpartum 171, 325, 459, and 501 respectively.

In addition to vitamin A, the carotene, protein, fat, ash, and total solids of each sample were determined. The results show that, while the supplements increased the vitamin A content of the milk, they had no effect on any of the other constituents named. Protein and ash decreased and fat increased as lactation progressed, in all groups of patients. Concerning the effect upon the carotene content of the milk, the human being thus differs from the cow, for which Deuel *et al.* (*J. Nutrition* **22,** *303* (*1941*); **23,** *567* (*1942*)) had demonstrated that increased ingestion of vitamin A caused a decrease in the carotene content of the milk. The authors are however careful to point out that the human diets were uncontrolled, and that variations in the carotene

content of different diets might have obscured an effect on the carotene content of the milk. No evidence was observed of any harmful effect of the vitamin A supplements upon the women who received them, or upon their infants.

Hrubetz, Deuel, and Hanley conclude that their data "should not be interpreted to mean that the feeding of large doses of vitamin A to pregnant and lactating women is either necessary or desirable." One may go further and suggest that it is definitely undesirable. Taking the figures for eleven to thirty days postpartum, a woman receiving no vitamin A supplement with an infant which weighed 5 kg. and required 600 calories daily would in the normal course be supplying 900 ml. daily of milk with a value of 65 calories per 100 ml. According to the authors this amount of milk would also supply on the average 2070 I. U. of vitamin A, plus about 60 I. U. of carotene. This amount on any standard so far used is ample. By taking 200,000 I. U. of vitamin A daily, a nursing mother at this period can expect to increase the vitamin A content of her milk almost fourfold. This increase, as well as being unnecessary, is obtained in a wasteful manner, since only about 3.0 per cent of the vitamin A fed appears in the milk. Thus these results appear to strengthen the conclusion drawn from animal experiments by Dann (*loc. cit.*) that "the best way of applying vitamin A therapy with the object of giving the child a reserve of vitamin A is to dose it directly."

VITAMIN B-COMPLEX AND CONDITIONED REFLEX EQUILIBRIUM

A number of clinicians have affirmed the existence of "marginal" vitamin deficiency states. Other investigators, however, remain unconvinced and some individuals have adopted the frank position that there is no deficiency state which is not clinically apparent in the classic sense. The subject is admittedly a difficult one. The symptoms attributed to marginal deficiencies are sufficiently vague and nonspecific that it is possible to doubt their reality. A depletion of body stores may often be demonstrated but that does not constitute proof that a physiologic inadequacy exists. It is most interesting, therefore, that objective data have now been presented which may be interpreted as demonstrating the existence of a marginal deficiency state in experimental animals.

Gantt and Wintrobe (*Federation Proceedings* **4**, *22* (*1945*)) employed in their experiments 4 dogs which had developed conditioned reflexes to different auditory stimuli. The diet employed was deficient in the B-vitamins but was supplemented by adequate amounts of these factors. After the animals had shown a 100 per cent differentiation between two tones for some months, the vitamin supplement was withheld. After four to fifteen days the animals showed an inability to differentiate between tones despite repeated practice. This condition became progressively worse but no other changes were noted during the two months of the deficiency. Renewed administration of the vitamin supplement then restored the ability to differentiate. After several months of perfect differentiation, the vitamins were again withheld and again there was an impairment of conditioned reflex equilibrium. This equilibrium was once again restored to normal by vitamin administration. The authors conclude: "These experiments suggest the need of daily adequate amounts of at least some of the vitamin B group for optimal mental performance, the requirement for which may be in excess of the need for physical health and muscular work."

There is a need for objective data concerning the possible existence of marginal

vitamin deficiencies. The experimental approach adopted here appears to be promising. If these results can be corroborated and extended, they will do much to resolve certain of the disagreements in this field.

EFFECT OF SOY FLOUR ON THE BIOLOGIC VALUE OF WHITE BREAD PROTEIN

That the biologic value of the proteins of white bread can be improved by relatively large additions of soy flour has been well established by the work of Johns and Finks (*Am. J. Physiol.* **55,** *455* (*1921*)), and Kon and Markuze (*Biochem. J.* **25,** *1476* (*1931*)). However, the addition of 15 per cent of soy flour changes the appearance and flavor of the bread so much that it is no longer acceptable to the average consumer at the present time. Volz, Forbes, Nelson, and Loosli (*J. Nutrition* **29,** *269* (*1945*)) noted that 5 per cent of soy flour can be used without appreciably altering the appearance or flavor, and therefore studied in some detail the effect of adding this proportion of soy flour. Jones and Divine (*Ibid.* **28,** *41* (*1944*)) had determined the effect of adding 5 per cent of soy flour on the biologic value of white flour, finding that it caused considerable improvement. Volz, Forbes, Nelson, and Loosli now extend this information by measuring the improvement, made by adding 5 per cent of soy flour, on the proteins of white bread made with 3 per cent whole milk solids.

Growth experiments were performed with rats, using diets containing the wheat bread and the wheat soy bread in such proportions that the protein content of each diet was 9.2 per cent. The diet was made up with sucrose, butterfat, soybean oil, salt mixture,and cellophane, and adequate vitamin supplements were fed. Under these conditions the wheat soy bread produced better growth when the food intakes of the rats on the two diets were equalized. The mean gains per gram of protein ingested were 0.97 g. and 1.17 g. body weight respectively, a difference which was shown statistically to be highly significant. When instead of the two breads, patent flour was compared with patent flour plus 5 per cent soy flour, a more marked difference was found in growth rates, as would be expected. The gains in body weight per gram protein ingested were 0.80 and 1.13 g. respectively. Biologic values of the proteins of the two breads were determined by the method of Mitchell (*J. Animal Sci.* **2,** *263* (*1943*)), using the same diets as in the growth experiments. Here again a highly significant difference was observed: the biologic value of the protein of the wheat bread was 43.3, and that of the wheat soy bread was 47.7.

Volz, Forbes, Nelson, and Loosli also analyzed thirty-three samples of breads purchased in the open market during July and August, 1942, previous to the publication of Food Distribution Order No. 1, by the War Food Administration, which required that white bread contain 3 to 4 per cent of milk solids. For these thirty-three samples the average content of nonfat milk solids was found to be 2.30 per cent. It was not higher on the average in those breads claimed to contain milk solids than in the others. It varied from 0.35 to 6.20 per cent, and about one fifth of the breads contained less than 1 per cent.

EXPERIMENTAL NICOTINIC ACID RESTRICTION WITH HUMAN SUBJECTS

An interesting study of the maintenance of human subjects on diets very low in nicotinic acid has been described by Briggs, Singal, and Sydenstricker (*J. Nutrition* **29,** *331* (*1945*)). Two subjects who had previously suffered from pellagra were hospitalized and

received a diet low in nicotinic acid for nine and forty-two weeks respectively. At the beginning of the period of observation, one showed signs of mild nicotinic acid deficiency but the other did not. The urinary excretion of products of nicotinic acid metabolism was measured daily, but the work was rendered difficult to perform and perhaps the results made less clearcut by the chemical methods which were chosen.

It is now clear that the simplest and most reliable way to follow the excretion of nicotinic acid end products in the urine is to measure the output of N′methylnicotinamide, the principal end product (*see Nutrition Reviews* **3,** *263* (*1945*)). However, Briggs, Singal, and Sydenstricker employed the earlier methods devised to measure trigonelline when this compound was believed to be the chief end product, together with the nicotinic acid in the free and easily hydrolyzed forms. This procedure has several disadvantages. Since it does include trigonelline it necessitates the use of a diet free from or low in trigonelline, and it is probably less precise than the measurement of N′methylnicotinamide. The twenty-four hour urine samples were analyzed for creatinine as an index of the completeness of collection.

The basal diet contained 25 g. lean beef, 120 g. unenriched flour, 50 g. hominy, 25 g. oatmeal, 20 g. cream, 25 g. lard, 40 g. nucoa, 150 g. syrup, synthetic fruit juice, and sugar and hard candy ad libitum. By analysis the daily food allowance contained 2.4 mg. of nicotinic acid. Small quantities of one or another supplementary food low in nicotinic acid (squash, potatoes, spaghetti, onions, and cabbage) were added to the basal diet at different times. On this basal diet without any of the supplements one subject excreted after the first three weeks quite regularly about 1 mg. of "trigonelline" daily. The other received supplements containing an additional 0.6 mg. nicotinic acid, and excreted about 2 mg. "trigonelline" daily. The test for N′methylnicotinamide (F_2) showed none in the urine during the period of dietary restriction, but there was a reappearance of this metabolite after test doses of nicotinamide were given. Following 500 mg. doses of nicotinamide, the increased excretion of "trigonelline" was equivalent to about 15 per cent of the dose in one subject, and between 5 and 10 per cent in the second. The authors consider that anything less than 15 per cent, with their technic, represents a deficiency of nicotinic acid.

While the results of these excretion tests are difficult to assess, they do suggest the presence of a definite nicotinic acid deficiency in the 2 subjects. The clinical observations indicated that each had minimal lesions of nicotinic acid deficiency at the start, which however did not develop significantly during the period of dietary restriction. Each of them had previously suffered from classic pellagra, and the question is therefore raised why they did not again develop pellagra on such a low intake of nicotinic acid when they entered the restriction period already showing signs of nicotinic acid deficiency. No certain answer can be given to this question, but we have grounds for certain speculations. The authors consider that the synthesis of nicotinamide by the intestinal flora may be one reason, and point out that when one subject took 4 g. sulfaguanidine daily for a week, a definite decrease in the excretion of "trigonelline" occurred. They also refer to the maize toxin theory, and suggest the possibility that corn itself may inhibit the intestinal biosynthesis of nicotinic acid. (Since they wrote, evidence favoring this suggestion has been presented by Krehl, Teply, Sarma, and Elvehjem (*Science* **101,** *489* (*1945.*))

In that case, they conclude "possibly we have observed all which should have been expected on a corn poor diet." One can agree that it is indeed remarkable that an intake of less than 3 mg. nicotinic acid daily did not produce pellagra in these subjects, even after exposure to ultraviolet light; and that if corn had been included in their basal diet, the disease may well have developed.

NOTES

Wartime Nutrition Programs for Industrial Workers

Goodhart and Pett (*Milbank Memorial Fund Quart.* **23**, *161 (1945)*) have presented a broad review of the wartime nutrition programs for industrial workers in Canada and the United States. These programs have centered around the provision of in-plant food services which encourage the best possible use of available foods, favorably influence the food habits of the worker, and improve labor-management relationships.

As a result of such programs it is stated that 50 per cent of the plants in the United States and 61 per cent of those in Canada have some "on-the-job" food facilities. The quality of the lunch selected by the worker has improved during the war period; in one plant the percentage of the workers choosing "good" lunches approximately doubled from June, 1943 to June, 1944.

In Canada these programs are supervised by a permanent agency, the Ministry of National Health and Welfare, with certain compulsory powers. In the United States the program is under the jurisdiction of a wartime agency, the War Food Administration, which has no legal authority to demand compliance with recommendations made in the conduct of its industrial nutrition program.

The Proceedings of the Nutrition Society

The readers of *Nutrition Reviews* will undoubtedly be interested to learn of a new journal and a relatively new society devoted to nutrition. The *Proceedings of the Nutrition Society* began publication in 1944; it is the official organ of The Nutrition Society, which held its inaugural meeting October 18, 1941, in Cambridge, England. This society was established "to provide a common meeting place for [British] workers in the fields of nutrition." A successful effort was made to draw clinicians into the group. There are two divisions of the Nutrition Society, one meeting in Scotland, the other in England. Each division has been holding meetings four times a year. Thus far the sessions have consisted largely of "conferences to discuss special themes [of nutritional interest], particularly those of importance during the war."

The scope of the programs of the Nutrition Society may be illustrated by the contents of a recent number (*Proc. Nutrition Soc.* **2**, *nos. 1 and 2 (1944)*). Four scientific meetings are reported. During the first of these, six papers on the subject of nutrition in pregnancy were read. These papers discussed factors affecting fetal development and birth weight, the functions of the placenta, and the effects of supplementary feeding in pregnancy. Particularly interesting was a discussion by R. M. Titmus of stillbirth rates and neonatal mortality. He gave statistics for England and Wales during the three years 1936 to 1938. Per 1000 births there were 39 stillbirths, 11 deaths in the first twenty-four hours, 20 deaths in the rest of the first month, and 27 deaths in the rest of the first year. A total of nearly 10 per cent of births failed to result in a child alive at the end of the first year. Evidence was given that favorable economic circumstances markedly decreased both stillbirths and infant mortality. There was as much as 100 per cent differential in the stillbirth rate between the best and the worst economic areas. Infant mortality was even more influenced by economic status, but this influence was less marked during the first month than later during the first year. Thus, compared with very well-to-do classes, the poorest population segments suffered 50 per cent more deaths among infants under 1 month of age, and over 400 per cent more deaths among infants 6 to 12 months of age. The inference is that although it is possible to reduce infant mortality at all ages by better care (improvement in medical attention, nutrition, housing, etc.) achievements in this direction are more striking in the older age groups. Two subsequent meetings were

devoted to discussion of the influence of diet on lactation and of nutrition in infancy. The fourth meeting covered the "effect of processing and handling on the composition, vitamin content, keeping quality, and culinary properties of fish."

The outstanding characteristics of the Nutrition Society are the frequency of its meetings, the well coordinated programs of very broad scope, and the efforts to embrace a membership drawn from a wide variety of basic trainings, all interested in some aspect of nutrition. This type of society appears to function very successfully in Britain. It is not clear whether it would be feasible to have such an organization on a national scale in the United States without its size becoming too cumbersome. It is conceivable, however, that smaller regional groups might reasonably be established along the same lines to very good purpose.

Food Yeast

Two editorial articles (*Brit. Med. J.* **2**, *668, 796 (1944)*) give an account of the interest taken in food yeast by British official circles. As reported previously (*Nutrition Reviews* **1**, *416 (1943)*), the Colonial Office has formed a company, Colonial Food Yeast, Ltd., to grow food yeast on a commercial scale in Jamaica. It is hoped by the Office that this venture will create a new market for sugar cane and syrup (the carbohydrate to be used in growing the yeast) and produce valuable quantities of protein and vitamins for the improvement of nutrition in the native colonial populations.

The yeast to be grown is Torulopsis utilis, which is preferred to bakers' or brewers' yeast because of the greater economy of manufacture, and the less objectionable flavor of the product. One hundred grams of the dry food yeast are reported to contain 43 g. of high quality protein, 2 mg. of thiamine, 5 mg. of riboflavin, and 40 mg. of nicotinic acid. It thus contains much less thiamine than brewers' yeast, but more riboflavin. The protein of the yeast is notably rich in lysine, so that it might find particular value in supplementing a diet made up largely of cereals, whose protein is often low in lysine. Since it can be produced at the factory for 12 cents per pound, it offers a cheap means of improving the diet of impoverished people. An output of 12 tons a day is the aim for the first factory to be built in Jamaica.

Such yeast might be used to increase the nutritional value of soups and stews, and might even afford at present a better and cheaper method of enriching white bread than the addition of synthetic vitamins. Since for 3 cents it would be possible to supply 50 g. of high quality protein and an adequate daily supply of the B-vitamins, such yeast may play an important part in nutrition in the future.

Research Fellowships

The Public Health Service has announced the creation of National Institute of Health research fellowships. The junior research fellowships are available to those holding master's degrees in the sciences (such as physics, chemistry, entomology, etc.) allied to public health, from an institution of recognized standing. The stipend is $2400 per annum. The senior research fellowships are available to those holding a doctor's degree in one of the sciences allied to public health. The stipend is $3000 per annum.

These fellowships offer an opportunity for study and research at the Institute or some other institution of higher learning, in association with highly trained specialists in the candidate's chosen field. Letters of inquiry should be addressed to The Director, National Institute of Health, Bethesda 14, Md.

RECOMMENDED DIETARY ALLOWANCES*

REVISED, 1945[1]

Food and Nutrition Board, National Research Council, Washington, D. C.

	CALORIES	PROTEIN	CALCIUM	IRON	VITAMIN A[2]	THIAMINE[3]	RIBOFLAVIN[3]	NIACIN (NICOTINIC ACID)[3]	ASCORBIC ACID	VITAMIN D
		grams	*grams*	*mg.*	*I.U.*	*mg.*	*mg.*	*mg.*	*mg.*	*I.U.*
Man (156 lbs., 70 kg.)										
Sedentary	2500	70	0.8	12[4]	5000	1.2	1.6	12	75	[5]
Moderately active	3000	70	0.8	12[4]	5000	1.5	2.0	15	75	[5]
Very active	4500	70	0.8	12[4]	5000	2.0	2.6	20	75	[5]
Woman (125 lbs., 56 kg.)										
Sedentary	2100	60	0.8	12	5000	1.1	1.5	11	70	[5]
Moderately active	2500	60	0.8	12	5000	1.2	1.6	12	70	[5]
Very active	3000	60	0.8	12	5000	1.5	2.0	15	70	[5]
Pregnancy (latter half)	2500[6]	85	1.5	15	6000	1.8	2.5	18	100	400–800
Lactation	3000	100	2.0	15	8000	2.0	3.0	20	150	400–800
Children up to 12 years[7]:										
Under 1 year[8]	100/2.2 lbs. (1 kg.)	3.5/2.2 lbs. (1 kg.)	1.0	6	1500	0.4	0.6	4	30	400–800
1–3 yrs. (29 lbs., 13 kg.)	1200	40	1.0	7	2000	0.6	0.9	6	35	400
4–6 yrs. (42 lbs., 19 kg.)	1600	50	1.0	8	2500	0.8	1.2	8	50	400
7–9 yrs. (55 lbs., 25 kg.)	2000	60	1.0	10	3500	1.0	1.5	10	60	400
10–12 yrs. (75 lbs., 34 kg.)	2500	70	1.2	12	4500	1.2	1.8	12	75	400
Children over 12 years[7]										
Girls, 13–15 yrs. (108 lbs., 49 kg.)	2600	80	1.3	15	5000	1.3	2.0	13	80	400
16–20 yrs. (119 lbs., 54 kg.)	2400	75	1.0	15	5000	1.2	1.8	12	80	400
Boys, 13–15 yrs. (103 lbs., 47 kg.)	3200	85	1.4	15	5000	1.5	2.0	15	90	400
16–20 yrs. 141 lbs., 64 kg.)	3800	100	1.4	15	6000	1.8	2.5	18	100	400

* A 1945 revision of "Recommended Dietary Allowances" (*Circular no.* **115** *(1943)*) has been prepared by the Food and Nutrition Board of the National Research Council. Several changes have been made in the tabulated data and footnotes, in conformity with new information and a desire to clarify the basis of certain recommendations. Permission was kindly granted to print the revised table with footnotes in the current issue of NUTRITION REVIEWS.

[1] Tentative goal toward which to aim in planning practical dietaries; can be met by a good diet with a variety of natural foods. Such a diet will also provide other minerals and vitamins, the requirements for which are less well known. This revision at press, August 1945, to be published as National Research Council Reprint and Circular Series No. 122 "Recommended Dietary Allowances, Revised, 1945."

[2] The allowance depends on the relative amounts of vitamin A and carotene. The allowances of the table are based on the premise that approximately two thirds of the vitamin A value of the average diet in this country is contributed by carotene and that carotene has half or less than half the value of vitamin A.

[3] For adults (except pregnant and lactating women) on diets supplying 2,000 calories or less, such as reducing diets, the allowances of thiamine, riboflavin, and niacin may be 1 mg., 1.5 mg. and 10 mg. respectively. The fact that figures are given for different calorie levels for thiamine, riboflavin, and niacin, does not imply that we can estimate the requirement of these factors within 500 calories, but they are added merely for simplicity of calculation. Other members of the B complex also are required,

though no values can be given. Foods supplying adequate thiamine, riboflavin, and niacin will tend to supply sufficient of the remaining B vitamins.

[4] There is evidence that the male adult needs little or no iron. The requirement will be provided if the diet is satisfactory in other respects.

[5] For persons who have no opportunity for exposure to clear sunshine and for elderly persons, the ingestion of small amounts of vitamin D may be desirable. Other adults probably have little need for vitamin D.

[6] During the latter part of pregnancy the allowance should increase approximately 20 per cent over the preceding level. The value of 2500 calories represents the allowance for pregnant, sedentary women.

[7] Allowances for children are based on the needs for the middle year in each group (as 2, 5, 8, etc.) and are for moderate activity and for average weight at the middle year of the age group.

[8] Needs of infants increase from month to month with size and activity. The amounts given are for approximately 6 to 8 months. The dietary requirements for some of the nutrients such as protein and calcium are less if derived largely from human milk.

Further recommendations:

Fat. There is available little information concerning the human requirement for fat. Fat allowances must be based at present more on food habits than on physiological requirements. While a requirement for certain unsaturated fatty acids (the linoleic and arachidonic acids of natural fats) has been amply demonstrated with experimental animals, the human need for these fatty acids is not known. In spite of the paucity of information on this subject there are several factors which make it desirable that fat be included in the diet to the extent of at least 20 to 25 per cent of the total calories and that the fat intake include "essential" unsaturated fatty acids to the extent of at least 1 per cent of the total calories. At higher levels of caloric expenditure, e.g., for a very active person consuming 4500 calories and for children and adolescent persons, it is desirable that 30 to 35 per cent of the total calories be derived from fat. Since foodstuffs such as meat, milk, cheese and nuts may be expected to contribute "invisible" fat to the extent of from one-half to two-thirds of the total amounts of fat implied by the above proportions of the total calories, it is satisfactory to use separated or "visible" fats such as butter, margarine, lard, and shortenings only to the extent of one-third to one-half of the amounts indicated.

Copper. The requirement for copper for adults is about 1 to 2 mg. daily. Infants and children require approximately 0.05 mg. for each kilogram of body weight. The requirement for copper is approximately one-tenth that for iron. A good diet normally will supply sufficient copper.

Iodine. The requirement for iodine is small, probably about 0.002 to 0.004 mg. daily for each kilogram of body weight, or a total of 0.15 to 0.30 mg. daily for the adult. This need is met by the regular use of iodized salt; its use is especially important in *adolescence* and *pregnancy*.

Phosphorus. Available evidence indicates that the phosphorus allowances should be at least equal to those for calcium in the diets of children and of women during the latter part of pregnancy and during lactation. In the case of other adults the phosphorus allowances should be approximately 1.5 times those for calcium. In general it is safe to assume that if the calcium and protein needs are met through common foods, the phosphorus requirement also will be covered, because the common foods richest in calcium and protein are also the best sources of phosphorus.

Vitamin K. The requirement for vitamin K usually is satisfied by any good diet. Special consideration needs to be given to newborn infants. Physicians commonly give vitamin K either to the mother before delivery or to the infant immediately after birth.

Salt. The needs for salt and for water are closely interrelated. A liberal allowance of sodium chloride for the adult is 5 grams daily, except for some persons who sweat profusely. The average normal intake of salt is 10 to 15 grams daily, an amount which meets the salt requirements for a water intake up to 4 liters daily. When sweating is excessive, one additional gram of salt should be consumed for each liter of water in excess of 4 liters daily. With heavy work or in hot climates 20 to 30 grams daily may be consumed with meals and in drinking water. Even then, most persons do not need more salt than usually occurs in prepared foods. It has been shown that after acclimatization persons produce sweat that contains only about 0.5 gram to the liter in contrast with a content of 2 to 3 grams for sweat of the unacclimatized person. Consequently after acclimatization, need for increase of salt beyond that of ordinary food disappears.

Water. A suitable allowance of water for adults is 2.5 liters daily in most instances. An ordinary standard for diverse persons is one milliliter for each calorie of food. Most of this quantity is contained in prepared foods. At work or in hot weather, requirements may reach 5 to 13 liters daily. Water should be allowed *ad libitum*, since sensations of thirst usually serve as adequate guides to intake except for infants and sick persons.

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